Spray Reagents

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Introduction

The detection of chromatographic zones on a developed thin-layer plate usually relies on the absorption or emission of electromagnetic radiation in the visible or ultraviolet range. Some compounds are visibly coloured, others absorb UV light or exhibit fluorescence when excited by UV or visible light, but most require visualization using an appropriate spraying or dipping reagent. Due to the inert nature of the adsorbents commonly used in thin-layer chromatography (TLC) layers, chemical reactions can be carried out *in situ* without destroying the adsorbent or binder characteristics. Many such detection reagents exist for TLC, and lists of formulations have been collated in a number of publications. Like a number of other features of the TLC procedure, this highlights the versatility and uniqueness of thin-layer chromatography compared with other chromatographic techniques. Often, quite aggressive reagents such as hydrochloric acid or sulfuric acid can be used to detect separated analytes *in situ*. Such reagents are included along with iodine vapour or nitric acid vapour as universal reagents that can be used to visualize a wide range of compounds of different types. Some of these can be termed destructive reagents, particularly those involving charring. Some reagents are much more specific for groups of compounds such as alcohols, aldehydes, ketones, esters or acids. These are termed group-specific reagents. With regard to specificity, this is about the limit of what is possible as no genuine substance-specific reagents exist. When no irreversible chemical reaction is used for detection on the chromatographic layer, the form of visualization is termed non-destructive. Included in non-destructive techniques are visible and UV light, and sometimes the use of iodine or ammonia vapour. The latter two reagents are included as in many cases the 'reaction' is reversible.

Often, separated compounds can be detected and visualized by a combination of the above techniques. A non-destructive technique may be used initially, followed by a universal reagent, and then finally a group-specific reagent to enhance selectivity and sensitivity. Often, for a particular analyte there may be several visualization reagents available, but usually there is a noticeable difference in sensitivity of detection between them. Also stability may play an important part in the selection of a suitable detection reagent. Some reagents have good stability over a number of weeks, however, there are those that must be made up fresh and used almost immediately. The visualized chromatographic zones may also differ in stability. Some may fade quite quickly, whilst others, although remaining stable, become more difficult to visualize as the background darkens or is affected in some other way by the reagent. Fortunately, the majority of reagents do give acceptably stable results. Sometimes dark or coloured backgrounds can be lightened by exposure of the chromatographic layer to acidic or alkaline vapours. However, all these effects will need to be taken into consideration so that the most effective visualization procedure is used.

After visualization, further analysis can be performed by *in situ* spectrodensitometric scanning in the absorbance, fluorescence quenching, or fluorescence modes, in addition to removing some or all of the separated chromatographic zone for further analysis by infrared (IR), Raman, nuclear magnetic resonance (NMR), mass spectrometry (MS) or radiography. Using these hyphenated techniques, more useful analytical data can be obtained. In fact, the stability of the layers and the detected chromophores often allow useful data to be collected even if the TLC plate had been developed some days or weeks previously.

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Non-destructive Methods

Visible Spectrum

Some compounds are sufficiently coloured, for example natural and synthetic dyes, and nitrophenols, to give an absorption in the visible part of the electromagnetic spectrum. However, it is the remaining part of the visible radiation that is reflected and is seen by either the naked eye or light-detecting equipment. Prechromatographic derivatization can often be used to improve resolution of the analytes of interest, but also make them visible to the naked eye. One example of this is the formation of yellow dinitrophenylhydrazones of ascorbic acid and its homologues.

Ultraviolet Spectrum

Colourless separated components on the TLC/high performance (HP) TLC layer may absorb electromagnetic radiation at shorter wavelengths than the visible spectrum. These are detected in the UV range, normally at 400–200 nm. Such compounds are detected using

UV-sensitive detectors, e.g. photomultipliers. Often exposure to UV light at short-wave radiation (254 nm) or long-wave radiation (366 nm) is all that is necessary for absorbing or fluorescing substances to be observed. Most manufacturers' UV lamps and cabinets function at either or both of these wavelengths.

To aid absorbance, many commercial pre-coated TLC layers contain an inorganic phosphorescent indicator or an organic fluorescent indicator. Inorganic indicators used include uranyl acetate (yellow–green), manganese zinc silicate, zinc cadmium sulfide, zinc silicate (green) and tin strontium phosphate (blue). Nearly all silica gel pre-coated plates and sheets contain this type of indicator. Most indicators exhibit a bright green, yellow or blue phosphorescence when excited by UV light at 254 or 366 nm.

Detection by absorbance in these cases relies on the phosphorescence being quenched by the sample components. This process is known as fluorescence quenching because the phosphorescence is very short lived. Hence, the effect is best observed by continual exposure to UV light. In most cases, pink or violet spots/bands are observed for quenching on the green phosphorescent background, whereas grey–black spots/bands are usually seen on the blue background. In most applications, the inorganic indicators are quite stable with little or no elution from developing solvents, and remain unaffected by most dyeing reagents and the temperatures used to effect reactions.

Organic fluorescent indictors can also be used. Most of these fluoresce in long-wavelength UV light (366 nm) and are coded F_{366} or UV_{366} by the manufacturer. Most cellulose pre-coated layers contain this type of indicator. Substances used include optical brighteners, hydroxypyrene sulfonates, fluorescein and rhodamine dyes. Some pre-coated plates are manufactured with both types of indicator present to give possible quenching by sample components at both wavelengths.

The process of fluorescence quenching is caused by the electromagnetic radiation providing the energy to bring about an electronic transition from the ground state to an excited singlet state. As the excited electrons return to the ground state, they emit the energy at a longer wavelength, usually in the visible range. Phophorescence quenching is slightly different. Rather than returning directly to the ground state, the electrons enter an excited triplet state and then return from there to the ground state. As before, the energy is emitted at a longer wavelength. However, as the decay is longer the process is better described as phophorescence rather than fluorescence.

Many analytes, however, either absorb insufficiently or not at all by these techniques. In these instances suitable detection reagents are used to give coloured spots/bands *in situ*. If these are reversible reactions, then they can still be termed non-destructive techniques.

Reversible Reactions

Iodine Vapour

Iodine is a very useful universal reagent, but it should never be overlooked that some reactions with iodine are non-reversible (discussed later in Non-reversible Reactions). The use of iodine as vapour enables the detection of separated substances rapidly and economically before final characterization with a group-specific reagent. Where lipophilic zones are present on a silica gel layer, the iodine molecules will concentrate in the substance zones giving brown chromatographic zones on a yellow background.

Preparation of the reagent simply involves putting a few iodine crystals in a dry chromatography chamber, replacing the lid and allowing the iodine vapour to fill the tank for a few hours. The developed chromatogram is then introduced into the chamber and as soon as the chromatographic zones are recognized, the layer is removed and the results documented. The adsorbed iodine can then be allowed to evaporate from the surface of the layer in a fume cupboard. The chromatogram can then be subjected to further reactions. If more permanent results of the iodine impregnation are required, then the chromatographic zones can be sprayed or dipped in a starch solution (0.5–1%) to give blue starch–iodine inclusion compounds.

Iodine detection works well on silica gel 60 and aluminium oxide layers. However, results are usually poor on reversed-phase layers as the lipophilicity of the layer does not differ appreciably from the substance zones.

Iodine vapour reversible reactions occur with a wide range of organic lipophilic molecules, e.g. hydrocarbons, fats, waxes, some fatty acids and esters, steroids, antioxidants, detergents, emulsifiers, antibiotics and many miscellaneous pharmaceuticals.

Ammonia Vapour

Ammonia vapour is often employed in improving the sensitivity of visualization of organic acids where pH indicators like bromocresol green or bromophenol blue have been used initially. The presence of ammonia has the effect of giving a sharper contrast between the chromatographic zones and the plate background. The process of exposure to ammonia vapour can be carried out simply by holding the chromatographic plate face down over a beaker of strong ammonia solution. However, it can be done more

elegantly by pouring ammonia solution into one compartment of a twin trough developing tank and placing the TLC plate in the dry compartment. With the lid in place the TLC plate is exposed to an almost even concentration of vapour. The process is reversible with time as the ammonia soon evaporates from the adsorbent surface.

Non-reversible Reactions

Destructive Techniques

Most post-chromatographic chemical reactions on the layer could be described as 'destructive' in that a chemical reaction has taken place and visualized compounds are no longer those that were applied as the sample. However, in order that a clear distinction can be seen between a truly destructive technique and a chemical derivatization or other chemical reaction, the destructive techniques described here will be limited to charring and thermal activation.

Charring techniques Charring usually involves treatment of the developed chromatogram with sulfuric acid and then heating at temperatures of 100–180°C for 5–20 min. The reaction is fairly non-specific, detecting most substances that are organic in nature as carbon deposits. Hence, sulfuric acid can also be termed a 'universal reagent'. Sometimes fluorescent chromatographic zones are produced at temperatures below 120°C, but their intensities are very dependent on the time of heating. Most charring occurs at higher temperatures, typically 150–180°C.

Although sulfuric acid charring is a relatively easy technique, there are limitations. Overheating of manufactured pre-coated layers results in the whole layer turning grey or even black. The problem is that commercial binders in pre-coated layers will also char under extreme heat in the presence of sulfuric acid. Most sulfuric acid reagents for dipping and spraying commercial TLC plates consist of a 10–20% v/v solution in water/methanol. Charring reactions will also occur sometimes with other acids, e.g. phosphoric acid. In conjunction with copper acetate, lipids can be detected by charring at 180°C.

A further point to bear in mind is the care that needs to be taken if charring techniques are used in conjunction with reversed-phase silica gel layers. Due to the carbon loading, excessive heating will cause a dark or even black background to appear.

Thermal activation techniques It has been observed that heating some substances to high temperatures on certain chromatographic adsorbents results in chromatographic zones that are fluorescent under exposure to UV light. This process is known as thermochemical activation. The moderately polar stationary phase, a NH_2-bonded silica gel layer, gives the best results for this type of detection.

It has been postulated that under the catalytic influence of the surface of the silica gel bonded layer, functional groups are eliminated and aromatic ring systems develop, which are excited by long wavelength UV light at 366 nm. In general, compounds with heteroatoms (nitrogen, oxygen, sulfur and phosphorus) more readily undergo this reaction than pure hydrocarbons. Under the influence of the catalytic adsorbent surface, substances rich in π-electrons are formed that conjugate to produce products that are fluorescent when appropriately excited. Changes in pH can alter the excitation and fluorescing wavelengths.

The derivatives formed remain stable for weeks, and the fluorescence can frequently be intensified and stabilized by dipping the chromatogram in organic solvent solutions of Triton X100, liquid paraffin and polyethylene glycol. If the NH_2-bonded layer contains a fluorescent indicator (F_{254}), then often appreciable fluorescence quenching can occur under UV light at 254 nm. Sometimes compounds that only weakly fluoresce can exhibit strong fluorescent absorption (e.g. vanillic acid and homovanillic acid). Thermal activation works well for the detection of catecholamines, fruit acids and some carbohydrates.

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Chemical Reactions on the Layer

Most chemical reactions on the planar chromatographic layer are carried out after development of the chromatogram. This is described as post-chromatographic visualization and is particularly useful when the presence of 'unknowns' in the sample need to be detected. However, it is also possible, as will be shown later, to carry out a pre-chromatographic derivatization before chromatographic development and this can have significant advantages. As post-chromatographic visualization is the major method of detection, it is this technique that will be given consideration first.

Post-chromatographic visualization Post-chromatographic identification of chromatographic zones can be achieved by spraying or dipping the TLC/HPTLC layer with a universal or group-specific reagent. Sometimes 'destaining' techniques can improve sensitivity where such reagents are employed. Where complex mixtures of large numbers of analytes of differing functionality are involved, sequencing reactions can be used to locate groups of compounds progressively.

The most used and dependable reagents are discussed in detail below, however, this represents only a small fraction of the detection reagents available, and the literature should be consulted for a more comprehensive review. In choosing the best detection reagent, the following points should be borne in mind:

1. Sensitivity of detection.
2. Selectivity of the reagent for the analytes of interest.
3. Background effects, particularly where plates are to be scanned spectrophotometrically.
4. Stability of detection reagent.
5. Stability of the chromatogram after chemical or thermal treatment.
6. Ease of preparation of the spraying or dipping reagent.
7. Hazards associated with the use of particular detection reagent.

Universal chemical reagents

Iodine vapour/solution Iodine reacts chemically with unsaturated compounds, which is rarely reversible on silica gel layers. Also irreversible oxidations, electrophilic substitutions, addition reactions, and the formation of charged transfer complexes have been observed. The so-called 'iodine reaction' is possibly an oxidation with the initial formation of a radical cation as shown in the following reaction equation:

$$R\colon + \tfrac{1}{2}I_2 \rightarrow R^{\cdot +} + I^-$$

A number of possible reaction pathways can then occur. **Table 1** lists some of the common reactions that take place on the chromatographic layer with iodine. Iodine also possesses fluorescence quenching properties, hence, chromatographic zones that contain iodine will appear as dark zones on a silica gel layer containing a fluorescent indicator when viewed under UV light at 254 nm.

In the case of both reversible and non-reversible reactions with iodine vapour, the chromatographic zones can be 'fixed' by further treatment with a 0.5–1.0% aqueous starch solution. The well-known deep blue iodine–starch complex is formed, which has good stability. As the reaction is very sensitive it is important to make sure that little iodine remains in the background, otherwise the whole background will be coloured blue!

The detection limits are usually a few micrograms of substance per chromatographic zone, but there are some cases where the detection is lower still (e.g. 200 ng glucose). Iodine may also be applied as a solution and is usually prepared in an organic solvent, such as petroleum spirit, acetone, methanol, chloroform or diethyl ether. A suitable dipping solution would be 250 mg iodine in 100 mL petroleum spirit. Such solutions have the advantage that, in some cases, the iodine is enriched to a greater extent in the chromatographic zones when dissolved in a lipophilic environment than a hydrophobic one. Hence, the sensitivity can be improved.

Ammonia vapour Ammonia vapour has a number of uses in chromatographic zone detection:

1. When pH indicator solutions are used to detect aliphatic and aromatic carboxylic acids and fatty acids, ammonia vapour can help to intensify the colours against a contrasting background (often a reversible reaction).

Table 1 Examples of iodine reactions on the TLC layer with a common range of organic substances

Substance	*Reaction*
Polycyclic aromatic hydrocarbons, indole and quinoline derivatives	Formation of oxidation products
Quinine alkaloids, barbiturates and calciferol	Addition of iodine to the double bonds
Opiates, brucine, ketazone and trimethazone	Iodine addition to the tertiary nitrogen for the opiates Addition reaction with the $-OCH_3$ group of the brucine Ring-opening reaction in the case of the ketazone and the trimethazone
Thiols and thioethers	Oxidation of sulfur and addition across the double bond in the thiazole ring
Alkaloids, phenthiazines and sulfonamides	Complex formation

2. There are a number of instances where residual chemical detection reagent gives a background colour. Often exposure to ammonia vapour has the apparent effect of bleaching it from the layer. (An example of this is in the use of molybdophosphoric acid reagent, where the background yellow colour is removed.) This effect increases sensitivity of detection.
3. Stabilization of some reactions on the layer is also possible with ammonia vapour. An instance of this is the blue colour of tryptamine after reaction with Gibb's reagent.

Nitric acid vapour Most aromatic compounds can be nitrated with the fumes from fuming nitric acid. The reaction works best if the developed chromatogram is heated to about 160°C for 10 min and introduced whilst still hot into a chamber containing the nitric acid vapour. Generally the chromatographic zones are rendered yellow or brown in colour. They also absorb in UV light at 270 nm. Some substances such as sugars, xanthine derivatives, testosterone and ephedrine fluoresce yellow or blue when excited by long wavelength UV light after such treatment.

Group specific reagents Many reagents give specific reactions with certain organic chemical groups and are called group-specific reagents. In most cases, the reaction mechanism is clearly understood. As a general rule these reagents are very sensitive. It is these reagents that make up the major part of the detection reagent lists that are readily available in a large number of TLC publications. The relative merits of a few of the major group-specific reagents are discussed below.

Oxidation/reduction reactions Often the most frequently used visualization techniques, oxidation/reduction reactions are group-specific depending on the particular reagent used. Some examples of oxidation reactions used in TLC are as follows: Emerson reagent (4-aminoantipyrine–potassium hexacyanoferrate [III]) for detection of arylamines and phenols; phosphomolybdic acid reagent for lipids and some sterioids; chlorine–*o*-toluidine reagent for vitamin B_1, B_2, B_6 and triazines; chloramine T reagent for steroids and purine derivatives; and chlorine–potassium iodide–starch reagent for amino, imino and amido groups, and triazine herbicides. Examples of reduction reactions include: tin(II) chloride–4-dimethylaminobenzaldehyde reagent for the detection of aromatic nitrophenols; blue tetrazolium reagent for corticosteroids, Tillman's reagent (2,6-dichlorophenolindophenol) for organic acids including vitamin C; and silver nitrate–sodium hydroxide reagent for reducing sugars and sugar alcohols.

Hydrazone formation The reagent mainly employed for hydrazone formation is 2,4-dinitrophenylhydrazine in acidic solution, which provides a specific reagent for carbonyl compounds (aldehydes, ketones and carbohydrates). Yellow or orange–yellow hydrazones, or osazones in the case of carbohydrates, are formed on the chromatogram. Ascorbic acid and dehydroascorbic acid also respond to this reagent. The sensitivity limit is of the order of 10 ng per chromatographic zone.

Dansylation Dansyl chloride and other derivatives are used to produce fluorescent dansyl derivatives of amino acids, primary and secondary amines, fatty acids and phenols. The dansylation of fatty acids is indirect as the acid amides must be formed first. This conversion is readily achieved with the reagent dicyclohexylcarbodiimide. In the next step, dansyl cadaverine or dansyl piperidine is used to form fluorescent derivatives of the amides. The detection limit is 1–2 ng fatty acids.

Diazotization Azo dyes are strongly coloured and can be produced on the TLC layer by reduction to primary aryl amines, diazotization and coupling with phenols. Conversely, phenols can be detected by reaction with sulfanilic acid in the presence of sodium nitrite (Pauly's reagent). The coloured zones formed by such reactions are often stable for a period of months.

A few named reagents exist that rely upon a diazotization reaction to detect specific groups of compounds. Two well-known ones are the Bratton–Marshall reagent and Pauly's reagent. The Bratton–Marshall reagent consists of two spray solutions: the first, is sodium nitrite in acid to effect the diazotization; and the second is a mainly ethanolic solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride. This reagent is used specifically to visualize primary aromatic amines, sulfonamides and urea, and carbamate herbicides. Pauly's reagent is used to visualize phenols, amines, some carboxylic acids and imidazole derivatives.

Metal complexes The cations of a variety of transition metals are electron acceptors and are therefore capable of forming complexes with colourless organic compounds that are electron donors. Coloured complexes are the result caused by electron movement within the orbitals of the central metal ion. The most important of these metal ions that form chelates are Cu^{2+}, Fe^{3+} and Co^{2+}, which have particular affinity

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for compounds that contain oxygen and nitrogen. Examples of this type of chemistry are the biuret reaction with proteins (resulting in the formation of a reddish-violet complex) and the reaction of the Cu^{2+} ion with aromatic ethanolamines (to form blue-coloured chelates). In addition there is the formation of reddish-violet colours of phenolic compounds with the Fe^{3+} ion.

Schiff's base reaction The Schiff's base reaction is a group-specific reaction for aldehydes. The reaction usually occurs under basic conditions with aromatic amines to form a Schiff's base. Aniline is normally used to form a coloured anil or Schiff's base with an aldehyde. Carbohydrates can be visualized with 4-aminobenzoic acid with the formation of coloured and fluorescent Schiff's bases. A similar reaction mechanism occurs with 2-aminobiphenyl for aldehyde detection. One of the most sensitive reagents for reducing sugar visualization, the aniline phthalate reagent, is also a Schiff's base reaction. The limit of sensitivity is 10 μg per chromatographic zone.

Other reactions There are a number of less well-used reactions such as halogenation with bromine or chlorine vapour, esterification of alcohols, hydrolysis reactions, and the formation of charge transfer complexes. Many other popular reagents do not fit into the above categories, yet they do constitute a major part of visualization reagent lists. For some of these, the reaction mechanism has not been fully elucidated. **Table 2** lists a selection of visualization reagents together with the classes of compounds visualized.

Sequencing reactions If it is known that particular functional groups may be present in the separated chromatographic zones, then reactions can be exploited more specifically, not necessarily to give direct identification, but to increase the evidence of the presence or absence of particular analytes. Here, specific reagent sequences can be used to give a wealth of evidence visually. Sequencing reactions are particularly useful where a number of differing functional group compounds are present on a chromatogram. An example illustrating the use of four well-known detection reagents is shown in **Figure 1**.

Where excess of one reagent has been used that may then interfere with the next reagent in the sequence, washing or 'destaining' steps will be necessary. Rinsing troughs in the form of dipping chambers can be used. Such sequential reactions are always carried out either to prepare a substance for a colour reaction that will follow later or to increase the amount of information that is obtained by exploiting a combination of different independent reactions. Therefore, information is provided that could not be obtained using a single reagent step.

Pre-chromatographic derivatization to enhance detection Group-specific reagents can prove very useful not only in detection but also in determining to some extent the choice of mobile phase for the development of the separation. If we are looking to determine the concentration of a particular compound, or similar compounds with the same functional group in a complex mixture, then prechromatographic derivatization with a group-specific reagent is an effective way of accomplishing this. The derivatization reaction is normally carried out before application of the sample to the chromatographic layer. Lengthy extraction procedures on sample preparation columns to clean up the sample before chromatography are not necessary. Such derivatizations are simple 'test tube' reactions, which usually are not time consuming.

Table 2 Popular visualization reagents for TLC

Visualization reagent	*Compound groups visualized*
Anisaldehyde–sulfuric acid	Terpenes, steroids, glycosides
Berberine	Fatty acids, triglycerides
Copper(II) acetate–phosphoric acid	Lipids, prostaglandins
Diphenylboric acid–2-aminoethyl ester	Flavonoids, carbohydrates
Dragendorff reagent	Alkaloids and other nitrogen-containing compounds
Ehrlich's reagent	Amines, indoles
Folin and Ciocalteu reagent	Phenols
Gibb's reagent	Phenols, indoles, thiols, barbiturates
Ninhydrin	Amino acids, peptides, amines, amino-sugars
Pinacriptol yellow	Alkyl and aryl sulfonic acids
Potassium hexaiodoplatinate	Alkaloids, nitrogen compounds, thiols
Thymol–sulfuric acid	Sugars, sugar alcohols
Vanillin–sulfuric acid	Essential oils, steroids

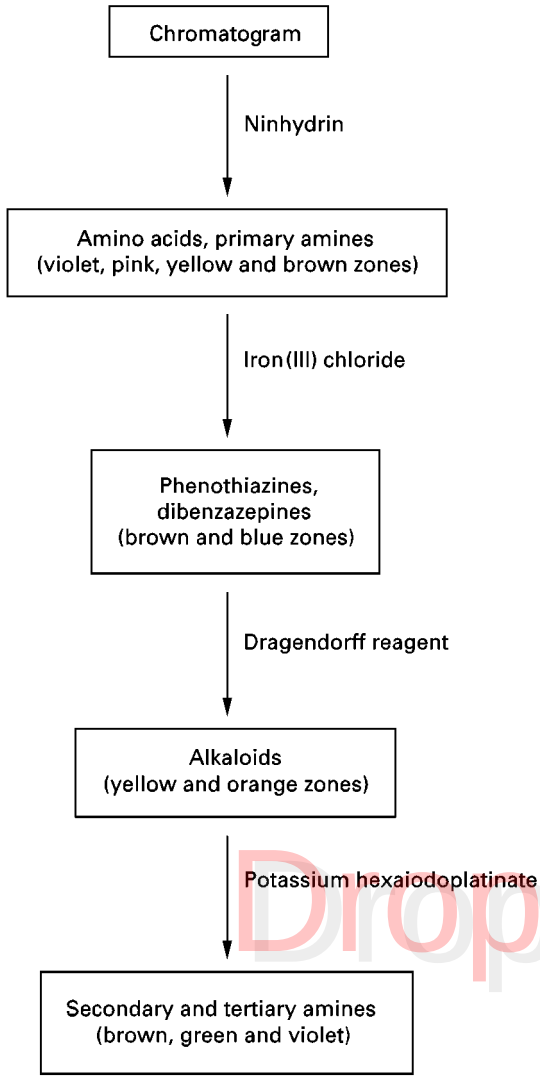

Figure 1 A typical visualization sequence for a TLC chromatogram. The sequence enables the identification of a number of different groups of nitrogen function organic compounds.

An example of such an analytical procedure is the determination of vitamin C in fruit juices. Although it is possible to apply the fruit juices directly to the TLC/HPTLC layer, develop the plate in a suitable solvent mixture, and then use a detection reagent to locate the vitamin C, a more precise and better resolution of the vitamin C can be achieved by derivatizing the sample first with 2,4-dinitrophenylhydrazine to form hydrazones with the keto groups (>C=O) present. The sample applied to the plate is then observable by its yellow colour. Using an appropriate solvent mixture for development, the yellow chromatographic zones can be visually observed as they separate and become resolved from each other.

An even more elegant and simple way to achieve prechromatographic derivatization is to carry out the reaction on the layer. At the point where the sample is to be applied on the chromatographic layer, the derivatization reagents can also be applied. Either the sample can be applied to the layer first, followed by the derivatization reagent shortly afterwards, or it can be done in the reverse order. The advantage of applying the derivatization reagent first is that a complete track across the width of the adsorbent layer can be applied, resulting in complete reaction with the sample when it is dosed, usually as bands, on top of the derivatization reagent. After appropriate drying and reaction time, development of the chromatogram can proceed using a solvent mixture that takes into consideration the polarity of the newly formed compounds.

It is also possible to carry out such 'functional chromatography' within the framework of 2-dimensional separations. The first ascending development is carried out in the usual way with underivatized sample. Before the second development, the separation track is subjected to treatment with a detection reagent specific to the functional group present in the substances (e.g. acids can be esterified, alcohols can be oxidized to ketones or aldehydes, carbonyls can be reduced to alcohols, carbonyls can form hydrazones or semicarbazones). The second development then follows in the usual way at 90° to the first.

Spraying Versus Dipping

Reagents can be applied to TLC/HPTLC plates either by dipping or spraying. The advantages and disadvantages of these approaches are considered below.

Spray Devices

A number of spray devices have been in use for many years. Perhaps the oldest is the atomizer spray, an all-glass unit that uses a rubber bulb to hand pump air into the main unit, which disturbs the detection reagent sufficiently to provide a fine spray out of the exit nozzle. More sophisticated versions have used propellant canisters to provide the means of atomizing the reagent solution into tiny droplets, which can then be evenly sprayed on to the chromatographic layer. As spray guns are relatively inexpensive, they tend to be the most popular spraying devices. However, it is important that the plastic components of the spray gun that come into contact with the detection reagents are solvent resistant. Spray canisters used to contain low-boiling CFCs as propellants, but now contain low-boiling, low-flash point hydrocarbons. More recently, rechargeable electro-pneumatically operated spray systems have become commercially available (an example is shown in **Figure 2**). The basic equipment consists of two spray heads with two different diameter capillaries, together with a charger and spray holder. The smaller diameter

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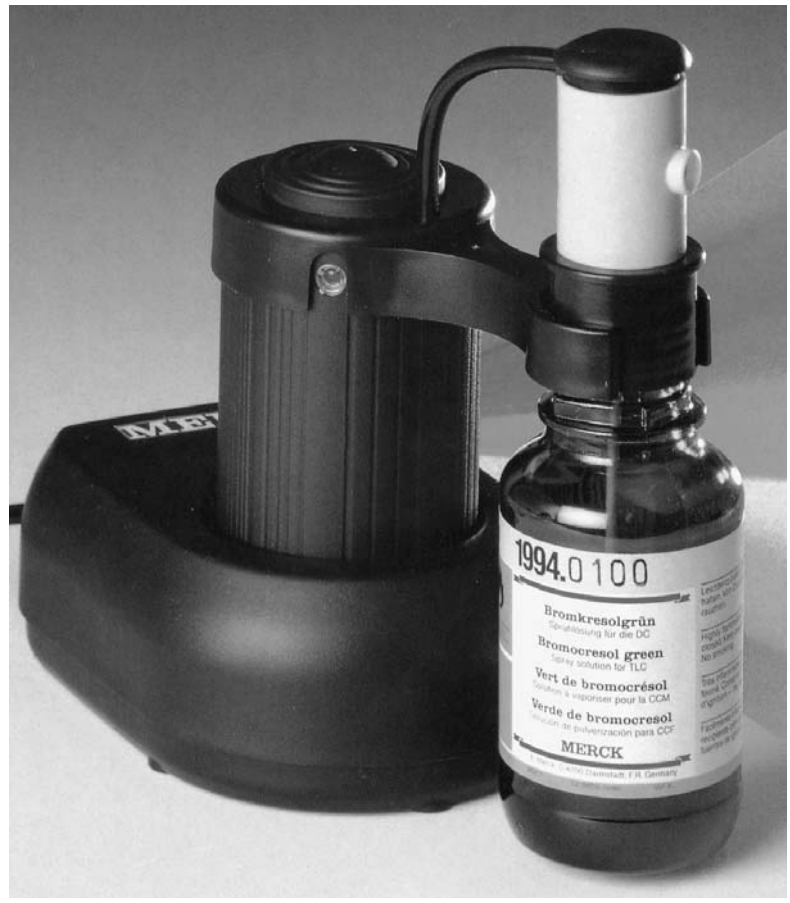

Figure 2 (See Colour Plate 33) An electropneumatically operated spray system for TLC. (Reproduced with the permission of Merck Ltd., UK.)

capillary (0.8 mm) is designed for spraying alcoholic or other low-viscosity solutions, whereas the larger diameter capillary (1.25 mm) is used for more viscous reagents like sulfuric acid. It can, of course, also be used to spray low viscosity reagents at higher rates.

Immersion Devices

In the last few years, dipping or immersion devices for TLC and HPTLC plates have been gaining in popularity. There are obviously benefits with regards to the handling of harmful, irritant or toxic chemicals, but also the uptake of reagent on to the chromatographic layer can be better controlled by immersion.

Chambers for immersion devices need to be as narrow as possible to limit the amount of reagent required, but not so narrow that the chromatographic layer is damaged by contact with the vessel. Manual dipping can be employed, but it is important that the chromatogram is fully dipped and the motion of dipping is done in one constant motion, otherwise 'tide' or 'contour'-like marks will appear on the layer. These will usually interfere with any further spectrodensitometric evaluation.

For good reproducibility and reliable constant uptake of reagent into the chromatographic layer, automated immersion devices are essential (an example is shown in **Figure 3**). These allow precise control of the time of immersion and the rate of dipping, which is essential for good reproducible results.

Future Prospects

The last decade has seen a move away from the use of spray reagents to immersion devices. This trend will inevitably continue as the hazards associated with the spraying of chemical reagents, many of which contain harmful substances, can be avoided by dipping. The greater control over the visualization process that dipping affords is also something that will be encouraged as the whole TLC technique becomes more standardized. Although many chemical reagents are already available, it is likely that new group-specific reagents will be discovered, often replacing those that contain toxic chemicals.

It is in the area of sequencing reactions where probably the greatest growth will occur as only a

Figure 3 A commercial immersion device for automatic dipping of TLC chromatograms in visualization reagents. (Reproduced with the permission of CAMAG, Switzerland.)

limited number of protocols are presently available. The visualization of the many different types of compounds present in herbal products may well drive the research required.

Pre-derivatization of sample on the TLC plate is proving to be a technique that often results in excellent resolution of analytes of interest. It is to be expected that further protocols will be developed in this area on the basis of reaction sequences that may already be available.

Tables showing the detection of compounds on TLC plates following chromatographic development can be found in appendix 17 on page 4801.

See Colour Plate 33.

See also: **II/Chromatography: Liquid:** Derivatization. **Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Instrumentation; Mass Spectrometry. **III/Thin-Layer Chromatography-Vibration Spectroscopy. Appendix 2/Thin-Layer (Planar) Chromatography: Detection.**

Further Reading

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Theory of Thin-Layer (Planar) Chromatography

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Introduction

Thin-layer chromatography (TLC) is a simple technique, many runs can be performed at the same time on a single plate, no detection problems occur provided a suitable reagent is used and moreover all solutes are detected. The main drawback is that a good detecting device designed for quantitative work is as expensive as a complete HPLC apparatus.

The observed decline of TLC is mainly due to the belief that efficiency is poor. To perpetuate that idea, qualitative data are obtained when no care is taken to carry out experiments! The advent of nanoplates coated with fine particles has given a renewed interest in the technique as excellent separations have been reported. By analogy to HPLC (high performance liquid chromatography) the acronym HPTLC (high performance thin-layer chromatography) appeared in the literature. Unfortunately different claims of performance have been published and some misunderstanding between pro and anti HPTLC camps have arisen, leading to fruitless discussions.

In their first paper, Ripphahn and Halpaap reported height equivalent to one theoretical plate (HETP) as low as 15 μm with these nanoplates; on the other hand Delley and Szekely cut a 'normal TLC' plate in four parts to yield the size of nanoplates and obtained identical results. The data of Ripphahn and